Original Article

Evaluation of salivary gamma-glutamyl transpeptidase as a biomarker in oral squamous cell carcinoma and precancerous lesions

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Abstract

Background: Oral cancer is such a common malignancy, but its manifestations are usually asymptomatic, and by the time the lesion is diagnosed its invasion is deep. This makes the survival rate poor and also the treatment rendered during such stages is extensive and debilitating. In this regard, a novel approach has been advocated in the estimation of biomarkers in the body fluids. Gamma-glutamyl transpeptidase (GGT)/ gamma-glutamyl transferase is an enzyme that is essential for the absorption of amino acids, especially in the degradation of glutathione. Its activity is increased in oral cancer and precancerous lesions.

Aims and Objectives: The purpose of this study was to assess the activity and concentration of GGT in precancerous and cancerous patients in comparison with normal patients and also to assess its efficacy as an effective tumor marker.

Materials and Methods: The study population comprised a total of 75 patients who were categorized into three groups as normal patients (25 cases in Group A), patients with precancerous lesions (25 patients in Group B) and patients with oral squamous cell carcinoma (25 cases in Group C). 5 ml of whole unstimulated saliva collection was done, it was centrifuged at 3000 rpm for 15 min and the supernatant thus obtained was used for the estimation of GGT levels. The detection was done by photometric method reading the absorbance at 405 nm.

Results: Group A patients had values of GGT ranging from 4 to 30U/L with a mean of 16.7 ± 1.94U/L. Group B had activity of GGT ranging from 39 to 65 U/L with a mean of 50.4 ± 1.67U/L. In group C, the evaluated GGT activity was between 53 and 86 U/L and the mean was 70 ± 2.37 U/L. Statistical analysis using Chi-square test was conducted. Correlations between Group A and Group B and between Groups A and C showed a statistically significant relation ($P < 0.005$).

Conclusion: Salivary gamma-glutamyl transferase activity can be used effectively as a tumor marker, and further studies with a larger sample size can be done to correlate this finding.

Keywords: Gamma-glutamyl transpeptidase/transferase, oral cancer, salivary enzyme marker

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INTRODUCTION

One of the major health concerns globally with regard to oral health is the development of oral cancer, and the most common among them is the occurrence of oral squamous cell carcinoma accounting to 90% of the cases.[1] This complex disease is a major cause of morbidity and mortality. Among the Indian population, the incidence is around 30%.[2] Precancerous lesions and conditions also show an increased risk for the development of malignancy. These are now designated as oral potentially malignant disorders by the World Health Organization.[3]

In spite of advancements in surgery, radiotherapy and chemotherapy, the mortality is still high. Furthermore, initiation of treatment following its early detection can dramatically improve the survival rate. Although the definitive diagnostic aid is biopsy, it is an invasive technique. Noninvasive techniques using salivary biomarkers can be of help in its early detection and monitoring. These molecular biomarkers reflect abnormal cell components, and the use of molecular medicine is profoundly changing the approach to diagnosis and treatment modality. Enzyme activity changes occurring in tissue, body fluids and serum can be potentially used as parameters in the diagnosis of some types of cancer.[4] Gamma-glutamyl transpeptidase (GGT) is a membrane-bound enzyme, which is not normally expressed in oral epithelial tissues.[5] Furthermore, GGT has been shown to increase considerably in various malignant tumors, precancerous lesions and conditions.[4,6] Thus, the present study was conducted to assess the activity of salivary GGT in normal, precancerous and cancerous patients and also to evaluate its role as a biomarker.

Aims and objectives

the present study was aimed with the following objectives:

- To evaluate the levels of salivary GGT in precancerous and cancerous patients in comparison with normal individuals
- To assess the salivary GGT as an effective biomarker.

MATERIALS AND METHODS

The study comprised 75 patients aged between 20 and 66 years. These individuals were categorized into three groups:

- Group A consisted of 25 patients with normal oral cavity findings without any lesion
- Group B comprised 25 patients with precancerous lesions, namely, leukoplakia, erythroplakia and oral submucous fibrosis (OSMF)
- Group C involved 25 patients with diagnosed cases of oral squamous cell carcinoma.

Inclusion criteria

In Group A, healthy individuals without any systemic diseases and in the age range of 20–65 years with normal oral cavity findings were included. In all Group B and C patients, the diagnosis was established based on history, clinical examination and histopathological findings.

Exclusion criteria

Group A patients with habits of tobacco or gutkha chewing and with any intraoral lesions were excluded. For all the three groups of the study, patients medically compromised with diabetes and liver diseases were excluded from the study after confirmation with obtained history, clinical examination and necessary laboratory investigations.

The individuals were explained about the purpose of the study, and informed consent was obtained from all patients. A volume of 5 ml of unstimulated whole saliva sample was collected using spitting method.[7] The patients were instructed to skip having any oral intake of food 2 hours before collection. The collection was done during hospital hours between 9.00 am to 10.00 am. The patients were also asked to rinse the oral cavity to remove any debris, and the sample was collected in a sterile test tube after 10 min. The collected salivary sample was subjected for GGT test by the Szasz methodology using Gamma GT kit. Saliva samples, thus, collected were subjected for the isolation of gamma-glutamyl transpeptidase enzyme, separation of GGT and its estimation. Gamma-GT (SL) kit contained reagents R_1 and R_2:

- R_1 reagent contains Tris buffer (133 mmol/L) and glycylglycine (138 mmol/L)
- R_2 reagent contains L-gamma-glutamyl -1-3-carboxy-p-nitroanilide as GLUPA-(23 mmol/L).

Enzyme preparation

All the saliva samples were centrifuged at 3500 rpm and were dialyzed. The dialyzed samples were again centrifuged at 3500 rpm for 15 min, and the supernatant obtained was used without concentration. The estimated assay activity was compared with the method of Sajjan *et al*.,[8] and it was found that there was no change in total enzyme activity. An enzyme solution was prepared by mixing 4 volumes of Reagent 1 with one volume of Reagent 2, and total working reagent of about 1000 μL was prepared. Sample of 100 μL was taken, mixed and incubated with working reagent at 37°C for 1 min. The amount of 5-Amino—2-nitrobenzoic acid released was detected using photometric method, reading the absorbance at 405 nm. One unit of enzyme activity is defined as that amount of the enzyme released to one nanomole 5-Amino—2-nitrobenzoic acid per min per ml of the enzyme solution under the assay conditions

employed. The specific activity was measured in units per liter of the protein (U/L).

RESULTS

Among Group A, 18 (72%) were male patients and 7 (28%) were female with an age range of 20–62 years and a mean age of 31.52 years. The recorded range of GGT value in Group A was 3.93–38.60U/L and the mean GGT value was 16.71 ± 1.94U/L (mean ± standard error) [Table 1].

In total 25 Group B patients with precancerous lesions, 23 (92%) were male and 2 (8%) were female with an age range of 23–64 years and a mean age of 38.8 years [Table 1]. Out of 25 patients, 12 (48%) were of OSMF, 10 (40%) had leukoplakia and 3 (12%) patients had erythroplakia [Table 2]. The recorded lowest value of GGT in the OSMF patient was 38.004U/L and the highest value was 65.19U/L in the patient with leukoplakia. The value range was between 38.004 and 65.19U/L [Table 1]. The mean GGT value obtained was 50.46 ± 1.67U/L. The obtained *P* value in comparison of Group A and Group B using statistical method of Chi-square test was less than 0.0005 [Table 3]. There was an increase of about three times (294%) with that of normal group. Thus, the value obtained was significant.

In Group C patients, 17 (68%) were male and 8 (32%) were female with an age range of 24–66 years and a mean age of 50.4 years [Table 1]. The youngest patient was 24 years with carcinoma of palate and the eldest patient was of 66 years having carcinoma of tongue. The recorded GGT values were 52.94–86.015U/L with a mean of 70.47 ± 2.34. Comparing the obtained GGT value statistically with Group A, $P < 0.0005$ [Table 4] and increase in GGT was about four times (417%). Furthermore, statistical comparison was done from Group B with Group C and the obtained *P* value was also significant [Table 5].

DISCUSSION

Oral squamous cell carcinoma is a common oral malignancy. It is usually asymptomatic for a longer duration of time. The manifestations are very late, and prompt treatment at an early stage can improve the quality of life of the patient. Among the diagnostic modalities, saliva has been used in the last 2000 years as related in the Chinese literature.[9] Since saliva has many advantages over the collection of blood, it is a very valuable diagnostic aid.[10,11] Therefore, estimation of tumor markers in saliva can predict the subsequent changes. Among the cellular changes occurring in oral cancer, changes in the GGT activity are also well known.[5]

GGT is a membrane-bound enzyme that is transiently expressed in fetal tissue, and in some adult cells, but not in normal oral epithelium. This enzyme transfers

Table 1: Depicting the gamma-glutamyl transpeptidase values in Group A, B and C patients

Serial number	Group A		Group B		Group C	
	Age/sex	GGT (U/L)	Age/sex	GGT (U/L)	Age/sex	GGT (U/L)
1	28/male	03.93	32/male	58.09	58/female	73.21
2	21/male	15.27	25/male	44.29	35/female	60.00
3	21/female	11.36	23/male	61.93	55/male	63.45
4	22/male	09.42	32/male	55.72	45/male	57.08
5	20/male	10.012	30/male	44.00	55/male	67.73
6	55/male	26.11	27/female	40.45	45/male	82.71
7	24/male	08.96	37/male	49.00	55/male	75.96
8	25/male	27.02	33/male	41.84	55/male	79.11
9	25/female	30.189	52/male	54.00	50/male	72.70
10	21/female	15.22	35/male	52.10	50/female	71.03
11	61/female	13.11	63/male	55.90	54/female	75.90
12	20/female	12.96	49/male	42.27	25/male	64.01
13	34/male	29.34	37/male	47.21	49/male	60.49
14	25/male	03.36	64/male	52.60	53/male	79.012
15	22/male	11.07	44/male	49.364	65/male	61.40
16	27/male	09.93	28/male	39.012	40/male	68.09
17	35/female	22.90	36/male	42.441	63/female	77.403
18	62/female	38.60	48/male	56.019	24/male	59.90
19	32/male	14.43	45/female	38.004	45/male	74.96
20	48/male	32.69	49/male	50.06	66/male	80.03
21	23/male	05.70	38/male	65.19	50/female	52.94
22	37/male	19.32	22/male	44.72	48/male	70.409
23	46/male	26.90	33/male	59.01	52/female	82.119
24	29/male	08.72	25/male	50.63	60/female	86.015
25	25/male	11.33	60/male	57.93	66/male	66.34

GGT: Gamma-glutamyl transpeptidase

Table 2: Depicts the number of diagnosed cases of potentially malignant disorders

Diagnosis	Number of cases (%)
Oral submucous fibrosis	12 (48)
Leukoplakia	10 (40)
Erythroplakia	3 (12)

Table 3: Statistical analysis of Group A and Group B

	Mean	SD	*P*	SE
Group B	50.4672	8.3742	0.0001	1.67
Group A	16.7140	9.7380		1.94

SD: Standard deviation, SE: Standard error

Table 4: Statistical analysis between Group A and Group C

	Mean	SD	*P*	SE
Group C	70.4704	11.7312	0.0001	2.34
Group A	16.7140	9.7380		1.94

SD: Standard deviation, SE: Standard error

Table 5: Statistical analysis between Group B and Group C

	Mean	SD	*P*	SE
Group B	50.4672	8.3742	0.0001	1.67
Group C	70.4704	11.7312		2.34

SD: Standard deviation, SE: Standard error

gamma-glutamyl residues to substrate. It is used in the synthesis of glutathione, which is necessary for amino acid absorption (Meister cycle).[12] The normal serum value of GGT is 10–30 IU/L. The metabolism of glutathione involves its intracellular synthesis and degradation. Glutathione also acts as a coenzyme. It reduces the oxygen toxicity by destroying the reactive oxygen compounds produced within cells. Degradation of glutathione takes place through the actions of gamma-glutamyl transpeptidase, gamma-glutamyl cyclotransferase, 5-oxoprolinase and dipeptidase. Renal GGT prevents excretion of glutathione from the body by initiating cleavage of this tripeptide into its constituent amino acids, which can then be reabsorbed.[13] GGT is a cell surface enzyme that hydrolyzes the gamma-glutamyl bond of extracellular reduced and oxidized glutathione, initiating their cleavage into glutamate, cysteine (cystine) and glycine. GGT is normally expressed on the apical surface of ducts and glands, salvaging the amino acids from glutathione in the ductal fluids.[14] GGT (now termed as gamma-glutamyl transferase) (EC2.3.2.2)[15] acts only on peptides or peptide-like compounds containing a terminal glutamate residue joined through terminal gamma carboxyl. Several human diseases are found to be associated with deficiency of specific enzymes in glutathione metabolism. High elevations of GGT are seen in primary or secondary (metastatic) neoplasm, alcoholic cirrhosis and prostatic malignancy. GGT catalyzes three types of reactions.

1. Transpeptidation: Gamma-glutamyl moiety is transferred to the acceptor
2. Autotranspeptidation: Gamma-glutamyl moiety is transferred to glutathione to form gamma-glutamyl glutathione
3. Hydrolysis: Gamma-glutamyl moiety is transferred to water.

The earliest estimation of salivary GGT was in patients with hepatobiliary and pancreatic diseases by Alonso *et al*.,[16] and they were of the opinion that salivary alkaline phosphatase and GGT could be used to differentiate between liver cirrhosis and liver tumor. GGT activity in frozen section samples in patients with oral squamous cell carcinoma was carried out by Calderon-Solt and Solt, and it was suggested that there was an expression of GGT in the sections, suggesting it to be employed as a marker.[17] Furthermore, histochemical examination of GGT in tobacco users and carcinoma patients by Mock *et al*.[6] showed focal aggregations of GGT positive cells, while diffuse activity was seen in dysplastic lesions. Gerson S J[5] suggested that GGT is not normally expressed in normal oral epithelium while expressed in tobacco users, suggesting it to be a valuable marker in the future prognosis. Our study was conducted based on the study by Sajjan *et al*.[8] where they observed an increased activity of salivary GGT in oral cavity carcinomas. They investigated the GGT isoenzymes in the submandibular salivary gland and concluded that the submandibular gland was the main source of salivary GGT. Transpeptidase electrophorentograms of the submandibular gland and saliva showed almost identical electrophoretic migration velocities. Based on these hypotheses, the present study was conducted. Our study showed a statistically significant rise of salivary GGT levels in precancerous and cancerous patients, which correlates with the findings of Sajjan *et al*.[8] This was done in comparison with normal subjects. Most of the studies conducted so far on GGT are on systemic diseases and its role in oral cancer detection is very limited. The present study showed elevated levels of GGT which are alike to previous studies, indicating that it can be one of the useful tumor markers in predicting the risk individuals.

CONCLUSION AND SCOPE

GGT is an early predictive marker for atherosclerosis, heart failure, arterial stiffness and plaque, gestational diabetes, various liver diseases including viral hepatitis, other infectious diseases and several life-threatening cancers.[18]

Thus, integration of all the previous study findings and observations from the present study strengthen the rationale

for the evaluation of salivary GGT in precancerous and cancerous conditions. It can be a reliable biomolecular marker in early detection and prevention of oral cancer. As collection of salivary samples is more easy and simple method in contrast to collection of blood samples, this could be routinely employed in dental clinics. The present study can be further reinforced using larger clinical samples, paving a way for further research in this area.

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Conflicts of interest

There are no conflicts of interest.

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